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Acetylcholinesterase gene expression in mammals including human

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ABSTRACT:

Acetylcholinesterase (AChE) is a crucial enzyme which deesterifies the ubiquitous neurotransmitter acetylcholine (ACh), thus inactivating it. In humans AChE activity is enclosed by a single gene, *ACHE* located within 6 kilobases of DNA sequence on chromosome 7q22 [1, 2]. Multiple forms of AChE protein arise from alternative splicing of exons found at the 3' end of the open reading frame [3]. The alternative splicing gives rise to carboxyl termini which differ in their capacity to disulfide bond with other catalytic and structural subunits or associate with the plasma membrane [4, 5]. Many functional units and components of *ACHE* are yet to be known at the gene level.

Keywords: Acetylcholinesterase, gene, expression, mammals, human

INTRODUCTION

The collagen-tailed or asymmetric forms (A) represent a major component of acetylcholinesterase (AChE) in the neuromuscular junction of higher vertebrates. One approach useful for investigating gene structure and expression of AChE gene has been given [6]. It relies on interspecies comparison of a single genetic locus. Genomic structure, alternative RNA splicing and amino acid sequences of genes encoding AChE in a wide variety of species have been reported. Promoter elements controlling expression of AChE genes in mammalian cells have been identified and studied functionally in mouse muscle [7]. Sequences of the human AChE 5'-flanking region and functional promoter elements or transcriptional regulation of AChE in mammalian cells are also partially known [8].

ACHE PROMOTER

Study of human cell lines defines the location of promoter region of the *ACHE* gene. The *ACHE* promoter includes a proximal 600 bp region essential for *ACHE* gene expression in benign tissues [8,6]. The proximal promoter includes among others, consensus motifs for the leukemia-associated factor AML1/Runx1 and *c-fos*, a transcription factor known to regulate *ACHE* gene expression under stress [9]. A distal enhancer domain of the human *ACHE* promoter located 17 kb upstream from the AChE transcription start site, includes functional binding sites for HNF3, a glucorticoid-response element and binding sites for NF-B, *c-fos* and C/EBP [10]. Transcriptional control of *ACHE* production depends also on an internal enhancer positioned within the first intron, which contains consensus-binding sites for AP2, SP1, NF-B and GATA1 (11). The transcriptional and post-transcriptional regulation of *ACHE* pre-mRNA is a neuro protection strategy but might involve long term damage [12]. Specifically variant-specific causal involvement of AChE in the progression of both neurodegenerative diseases (e.g. Alzheimer's and Parkinson's diseases) and neuromuscular syndromes (e.g. myasthenia gravis) raises the possibility that future therapeutic drugs might target specific *ACHE* variant(s) or the corresponding RNA transcripts.

EXPRESSION OF *ACHE* PROMOTER ACTIVITY

ACHE plays a critical role in apoptosis and its expression is regulated by Ca^{++} mobilization and showed that activated calpain, a cytosolic calcium-activated cysteine protease and calcineurin, a calcium dependent protein phosphatase regulate AChE expression during A23187-induced apoptosis [13]. The calpain inhibitor, calpeptin, and the calcineurin inhibitors, FK506 and cyclosporine A, inhibited acetylcholinesterase expression at both mRNA and protein levels and suppressed the activity of the human acetylcholinesterase promoter. In contrast, over-expression of constitutively active calcineurin significantly activated the acetylcholinesterase promoter. There is a significant role of the transcription factor NFAT (nuclear factor of activated T cells), a calcineurin target, in regulating the acetylcholinesterase promoter during ionophore-induced apoptosis. Over-expression of human NFATc3 and NFATc4 greatly increases the acetylcholinesterase promoter activity in HeLa cells treated with A23187. Over-expression of constitutive nuclear NFATc4 activates the acetylcholinesterase promoter independent of A23187, whereas over-expression of dominant-negative NFAT blocks A23187-induced acetylcholinesterase promoter activation.

EXPRESSION OF *ACHE* GENE

The human acetylcholinesterase coding sequence expression has been observed in various organisms. The human AChE shows expression in transgenic plants in its native GC-rich sequence. When compared to a matched sequence with (dicotyledonous) plant-optimized codon usage and a lower GC content. It shows a 5 to 10 fold increase in accumulation levels of the "synaptic" splice variant of human acetylcholinesterase in *Nicotiana benthamiana* plants [14]. This expressed the optimized gene as compared to the native human sequence. Transient expression assays and stable transformants demonstrated conspicuously increased accumulation levels. This increase is possibly due to the facilitated translation and reduced energy required to unfold the sequence-optimized mRNA. The codon usage differences may regulate gene expression at different levels and anticipate translational control of acetylcholinesterase gene expression in its native mammalian host as well.

Expression of *ACHE* gene in embryonic cells

Gene expression profiling demonstrates components of the cholinergic system, such as choline acetyltransferase, acetylcholinesterase and nicotinic acetylcholine receptors (nAChRs). They are expressed in embryonic stem cells and differentiating embryoid bodies (EBs) [15]. Triggering of nAChRs is expressed in EBs by nicotine. This resulted in activation of MAPK and shifts of spontaneous differentiation toward hemangioblast. *In viv*o, non-neural nAChRs are detected early during development in fetal sites of hematopoiesis. Similarly, *in vivo* exposure of the developing embryo to nicotine resulted in higher numbers of hematopoietic progenitors in fetal liver [15]. Nicotine stimulates the production of IL4 and IL5, implying a possible role of the cholinergic system in pathogenesis of allergic diseases. The nicotine-induced imbalance of the cholinergic system during gestation interferes with normal development. This provides the basis for negative health outcomes postpartum in active and passive smokers.

Expression of *ACHE* gene in endothelial cells

Acetylcholine is found in the nervous system and also in other cell types i.e. endothelium, lymphocytes, and epithelial and blood cells. This is also termed the non-neuronal cholinergic system. In a study [16] investigated the expression and subcellular localization of acetylcholinesterase (AChE) in endothelial cells. They significantly reported the expression of the 70-kDa AChE in both cytoplasmic and nuclear compartments. A nuclear and cytoskeleton-bound AChE isoform with approximately 55 kDa detected in endothelial cells is also known. This novel isoform is decreased in response to vascular endothelial growth factor via the proteosomes pathway. It is down-regulated in human leukemic T-cells as compared with normal T-cells. This suggests that the decreased expression of the 55-kDa AChE protein may contribute to an angiogenic response and associate with tumorigenesis. The involvement of 55-kDa AChE in different biological processes such as neural development, tumor progression, and angiogenesis has been established [16].

Expression of *ACHE* during muscle differentiation:

Acetylcholinesterase expression increases during muscle differentiation from myoblasts to myotubes. It reflect primarily a greater stability of the messenger RNA. Recently, the regulation of the acetylcholinesterase gene during early determination of the muscle phenotype has been found. To assess the role of the myogenic transcription factors in this regulation [7] employed the myogenic transcription factors to transform non-muscle cells into myoblasts in order. They analyzed the *Ache* promoter region by deletion analysis, point mutagenesis, and gel mobility shift assays. The myogenic transcription factors do not accelerate transcription of the *Ache* gene in spite of the presence of E-boxes at -335 base pairs from the start of transcription and in the first intron. They are not able to trigger stabilization of the *Ache* mRNA when constitutively expressed in 10T1/2 fibroblasts. A GC-rich region (at -105 to -59 base pairs from the start of transcription) containing overlapping binding sites for the transcription factors Sp1 and Egr-1 is essential for promoter activity. Mutation of the Sp1 sites reduces the promoter activity while mutation of the Egr-1 sites has little effect. Sp1 and Egr-1 compete for binding to overlapping sites and an increase in Egr-1 decreases the expression of the *Ache* gene.

EXPRESSION OF *ACHE* MRNA IN HUMAN

Human immune cells such as mononuclear leukocytes consisting of mainly T and B cells, bone marrow derived dendritic cells (DCs) and macrophages. They all express various nicotinic acetylcholine (ACh) receptor (nAChR) subunits (17). Activated T cells and DCs have the ability to synthesize ACh by choline acetyltransferase. This suggests the role of non-neuronal cholinergic system expressed in immune cells in the regulation of immune cell function. Stimulation of human leukemic T and B cell lines with nicotine causes a transient Ca(2+)-signaling. This is

antagonized by alpha-bungarotoxin, suggesting the involvement of alpha7 subunit. The alpha7 nAChRs have been shown to negatively regulate synthesis and release of tumor necrosis factor (TNF)-alpha in macrophages.

REGULATION OF PROTEIN OF *ACHE* IN MUSCLE DURING MYOGENIC DIFFERENTIATION

The transcriptional regulation of proline-rich membrane anchor (PRiMA), an anchoring protein of tetrameric globular form acetylcholinesterase (G(4) AChE), has been studied in muscle during myogenic differentiation under the influence of innervation [18].

During myotube formation of C2C12 cells, the expression of AChE(T) protein and the enzymatic activity increases. On the other hand the level of G(4) AChE was relatively decreased. This G(4) AChE in C2C12 cells was specifically recognized by anti-PRiMA antibody.

This suggests the association of this enzyme with PRiMA. Overexpression of PRiMA in C2C12 myotubes significantly increases the production of G(4) AChE. Calcitonin gene-related peptide, a known motor neuron-derived factor, and muscular activity were able to suppress PRiMA expression in muscle.

The suppression is mediated by the phosphorylation of a cAMP-responsive element-binding protein [18]. The expression of PRiMA, as well as PRiMA-associated G(4) AChE, in muscle is suppressed by muscle regulatory factors, muscular activity, and nerve-derived trophic factor(s).

***ACHE* GENE IN BRAIN TUMORS**

AChE is expressed in brain tumors specifically in meningiomas, astrocytomas and glioblastomas. Regulation of acetylcholinesterase (AChE) gene expression in human brain tumors, 3' splice variants of AChE mRNA and potentially relevant transcription factor mRNAs were labeled in primary astrocytomas and melanomas. Brain tumor-specific regulation of both expression and cellular retention of variant *ACHE* gene products has been revealed (19).

AChE expression in normal brain is regulated by c-fos and HNF3, under various stress conditions (9,10). This shows the ubiquitous nature of AChE expression patterns. The molecular origin of AChE's regulation in brain tumors is still unknown.

THE *ACHE* GENE IN MALIGNANCIES

ACHE gene modifications are common in leukemic patients. Such modifications in the wider population, and their consequences, if any are not yet known. The long arm of chromosome 7, where the *ACHE* gene resides is often modified in acute myeloid leukemia (AML), chemotherapy related myeloproliferative disorders [20], and tumors of neuronal and glial origin. Chromosome 7 anomalies in adult myelodisplasia and AML associate with poor prognosis. Anticholinesterase exposure which enhances *ACHE* gene expression increases the risk of leukemia [9]. The actual role of *ACHE* in tumorogenesis is not properly understood.

***ACHE* GENE IN STRESS**

Like many other stimuli, both stress and inhibition of AChE cause an increase in *ACHE* gene expression. That is associated with a shift in its pre-mRNA splicing pattern. Of the several variants of *ACHE* that arise due to alternative splicing. It is specifically the usually rare, soluble *ACHE*-R variant that is up-regulated [21]. Transgenic mice that over-express *ACHE* also show many of the same symptoms as stress, erratic behavior following circadian light/dark shift, progressive failure of learning and memory, intensified long-term potentiation (LTP) development of neuropathologies, progressive muscle fatigue and degeneration of neuromuscular junctions. Recent studies reports the extended promoter region of the ACHE gene containing both hematopoietic transcription factor binding sites and stress associated glucocorticoid response element half palindromic sites [12]. This soluble monomeric variant is expressed in embryonic and tumor cells in mammalian hematopoietic cells. It is induced in the brain under psychological, physical and chemical stress [22].

INHIBITION OF ACHE BY OP COMPOUNDS

Organophosphate (OP) compounds exert inhibition of cholinesterase (ChE) activity by irreversibly binding to the catalytic site of the enzymes. They are employed as insecticides for agricultural, gardening and indoor pest control. The biological function of the ChE enzymes is well known and has been studied since the beginning of the 20th century. In particular, acetylcholinesterase (AChE, E.C. 3.1.1.7) is an enzyme playing a key role in the modulation of neuromuscular impulse transmission [23]. The chosen organophosphates are the ones mainly used in Europe. Diazinon, chlorpyriphos, malathion, and phentoate, all of them are belonging to the thionophosphate chemical class.

The comparison between the effects of exposure on the developing embryos at different stages has been well studied. The identified biomarkers of effect for *in vitro* experiments were cell proliferation/apoptosis as well as cell differentiation. For in vivo experiments, the endpoints were developmental speed, size and shape of pre-gastrula embryos; developmental anomalies on neural tube, head, eye, heart. All these effects are mediated by ion channel activation, through the activation/inactivation of acetylcholine receptors (AChRs) [23].

MUTANT IN PROTEIN EXPRESSION IN THE PARATHION TREATED CELLS

Many studies have found an association between human cancer and exposure to agricultural pesticides such as the organophosphorous pesticides. Parathion is a cholinesterase inhibitor that induces the hydrolysis of body choline esters, including acetylcholine at cholinergic synapses. [24] have reported the etiology of breast cancers. The primary target of action in insects is the nervous system whereby pesticides inhibit the release of the enzyme acetylcholinesterase at the synaptic junction. Atropine is a parasympatholytic alkaloid used as an antidote to acetylcholinesterase inhibitors. The effect of parathion and atropine on cell transformation of human breast epithelial cells in vitro has been determined (24). The parathion is able to induce malignant transformation of an immortalized human breast epithelial cell line, MCF-10F as indicated by increased cell proliferation, anchorage independency and invasive capabilities. There is an increase in c-kit, Trio, Rho-A, Rac-3, EGFR, Notch-4, Dvl-2, Ezrin, beta catenin and mutant p53 protein expression in the parathion-treated cells. Atropine significantly inhibits this increase. In a human cell cycle array of 96 genes, 13 of them are altered by parathion treatment. Among the genes affected were the cyclins, such as cyclin D3, the cyclin-dependent kinases (CDKs) such as CDK41 and the minichromosome maintenance deficient (MCM) MCM2 and MCM3. The parathion influences human breast epithelial cell transformation and is an initiator factor in the transformation process in breast cancer.

CONCLUSION

The enzyme acetylcholinesterase (AChE) terminates synaptic transmission at cholinergic synapses by hydrolyzing the neurotransmitter acetylcholine. In addition, AChE is thought to play several 'non-classical' roles that do not require catalytic function. Most prominent among these is facilitation of neurite growth. The previously reported morphogenetic and proliferative properties of *ACHE* may be clues to its further understanding during morphogenesis, tumors, pesticide exposures etc. The behaviour and sensitivity of *ACHE* will also be an additional tool for knowing the expression of *ACHE* gene in mammals.

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